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Cloud Point Extraction and Spectrophotometric Determination of Amaranth in Food Samples Using Nonionic Surfactant Triton X-100 and Tetrabutylammonium Hydrogen Sulfate

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Cloud point extraction methodology was successfully employed for preconcentration of trace amounts of amaranth prior to its determination by spectrophotometry. The method was based on the extraction of amaranth as an ion pair with tetrabutylammonium ion from aqueous solution using Triton X-100 as non-ionic surfactant. The extracted surfactant rich phase was diluted with ethanol and its absorbance was measured at 518 nm by a spectophotometer. An optimum set of surfactant concentration, pH, equilibration temperature and time, tetrabutylammonium hydrogen sulfate and salt concentration were obtained. The calibration graph was linear in the range of 20-1600 ng ml^{-1} of amaranth in the initial solution with r = 0.9993 (n = 12). Detection limit based on three times the standard deviation of the blank ($3S_b$) was 13.0 ng ml^{-1} (n = 10), and the relative standard deviation (R.S.D) for 100 and 1000 ng ml^{-1} of amaranth was 4.2 and 1.4% (n = 10), respectively. The proposed procedure was applied to the determination of amaranth in different food samples.

Keywords: Amaranth, Triton X-100, Cloud point extraction, Spectrophotometry, Beverage, Jelly

INTRODUCTION

Food colorants have been used to improve appearance, taste, flavor and color of food stuffs in order to make them more attractive and appetizing. Synthetic dyes have been used instead of natural colors because of their high stability to light, oxygen and pH, relatively lower costs and color uniformity. Because some synthetic colors may be pathogenic, especially if they are consumed in excess, they are analyzed and evaluated by both the manufacturer and the organizations of health such as Food and Agricultural Organization (FAO) and World Health Organization (WHO). Amaranth is the most controversial of the color additives used. It had been used in foods since 1908 but is now banned from use in USA [1].

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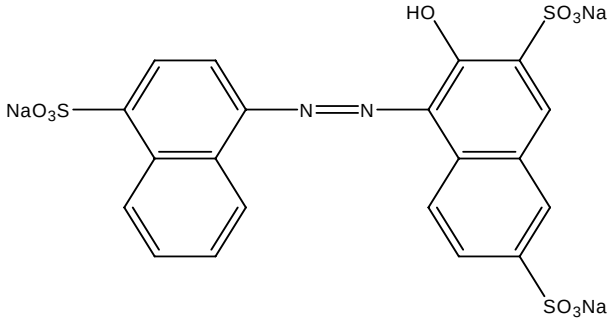

Fig. 1. Chemical structure of amaranth.

Amaranth (E123) is a purple-red synthetic coal tar or azo dye (Fig. 1), which, in addition to coloring in food, is used in dyeing and color photography. As amaranth is an azo dye, it is recommended that people who suffer from asthma or aspirin intolerance avoid it. Amaranth may also cause skin rash. Until

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1850, color was only obtained from natural origin, vegetal or animal such as extract of plants, trees, lichens, or insects. In 1856, Perkin synthesized the first dye. Most of synthetic dyes are azo dyes and are suspected to be carcinogenic. In 1970, Russian studies showed that amaranth was carcinogen. Nevertheless, amaranth is still used, as textile dyes for wool and silk as well as in photography and as food dye in caviar, sweets and beverages [2].

Amaranth has been determined in different samples by several methods such as spectrophotometry [3-5], voltammetry [6], capillary electrophoresis [1,7], square wave adsorption stripping voltammetry [8], thin layer chromatography [9], micellar electrokinetic capillary chromatography [10] and high performance liquid chromatography (HPLC) [11,12]. However, there is still of interest to develop simple methods for the determination of amaranth in different samples.

The aim of this study was the development of a cloud point extraction method for the determination of amaranth. The method is based on extraction of amaranth using nonionic surfactant Triton X-100 at pH 4.5.

EXPERIMENTAL

Apparatus

A GBC Cintra 101, UV-Vis spectrophotometer (Australia) was used for recording absorption spectra. The absorbance measurements were performed with a Jasco Model 7850 UV-Vis spectrophotometer (Japan) using 1cm glass cells. A Metrohm digital pH meter Model 632 (Switzerland) with a combined glass electrode was used to measure the pH. A Colora (England) thermostat bath, maintained at the desired temperature, was used for the cloud point temperature experiments.

Reagents

All chemicals used were of analytical grade and doubled distilled water was used throughout. A stock solution of 1000 μg ml^{-1} of amaranth was prepared by dissolving 0.1 g of the amaranth (Merck, Germany) in water and diluting to 100 ml in a volumetric flask. The desired concentrations were obtained by successive dilutions. A 0.5 M of Triton X-100 was prepared by dissolving 80.859 g of Triton X-100 (Aldrich) in water and diluting to 250 ml in a volumetric flask. A 0.01 M solution of tetrabutylammonium hydrogen sulfate was prepared by dissolving 0.849 g of $TBAHSO_4$ (Merck) in water and diluting to 250 ml in a volumetric flask. A solution of Na_2SO_4 (1.0 M) was prepared by dissolving 14.2 g of Na_2SO_4 (Merck) in water and diluting to 100 ml in a volumetric flask. A citrate buffer (pH 4.5) was prepared by dissolving 2.101 g of citric acid (Merck) in 100 ml of water and adding 0.1 M of NaOH to adjust the pH to 4.5 using a pH meter.

General Procedure

An aliquot of the amaranth solution, 5 ml of citrate buffer pH 4.5, 2 ml of 0.01 M of tetrabutylammonium hydrogen sulfate, 4 ml of 1 M of Na_2SO_4 and 2.5 ml of 0.5 M of Triton X-100 were added to a 50 ml volumetric flask and diluted to the mark with water. This solution was transferred to a 50 ml tube and placed in a thermostat bath at 80 °C for 30 min. The turbid solution was cooled in an ice bath in order to separate the phases. The aqueous phase was removed by decantation. The surfactant rich phase was diluted with ethanol in a 2 ml volumetric flask. The absorbance of the solution was measured at 518 nm.

RESULTS AND DISCUSSION

A cloud point extraction methodology was developed for preconcentration and spectrophotometric determination of amaranth. The absorption spectrum of amaranth was recorded after cloud point extraction with Triton X-100. It showed a maximum absorption band at 518 nm. Therefore, this wavelength was used for all the absorbance measurements. The effect of various cloud point extraction parameters such as pH, ternary salt, Triton X-100 and electrolyte concentration, equilibration temperature and time on the performance of the method was investigated in order to achieve highest sensitivity.

Effect of pH

For this study, the effect of a pH range of 1.5-7.0 on the cloud point extraction of 400 ng ml^{-1} of amaranth was investigated. As can be seen in Fig. 2, maximum absorbance was obtained at pH 4.5. Therefore, this pH was chosen for further work. Different buffer systems with pH 4.5 such as

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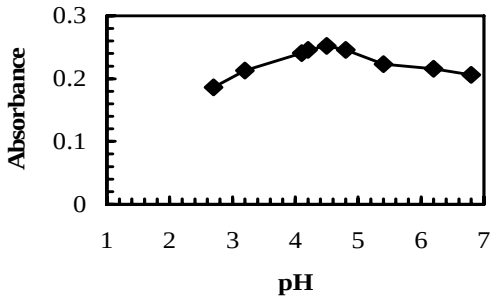

Fig. 2. The effect pH on the absorption of 400 ng ml^{-1} of amaranth after cloud point extraction with Triton X-100. Conditions: 0.025 M of Triton X-100; 4×10^{-4} M of $TBAHSO_4$; 0.08 M of Na_2SO_4; Temp. 80 °C; Time 30 min.

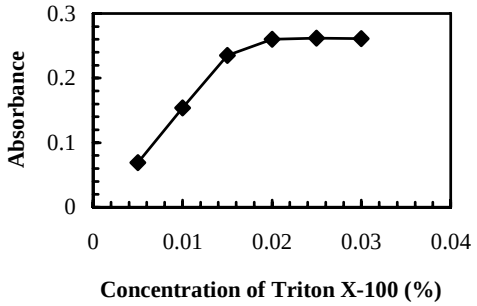

Fig. 3. The influence of Triton X-100 concentration on the absorption of 400 ng ml^{-1} of amaranth after cloud point extraction. Conditions: pH 4.5; 4×10^{-4} M of $TBAHSO_4$; 0.08 M of Na_2SO_4; Temp. 80 °C; Time 30 min.

citrate, phthalate and acetate were examined and citrate buffer was selected as the optimum for subsequent experiments because it did not change the absorbance of the solution after cloud point extraction. The effect of different volumes of citrate buffer solution (1-9 ml) was investigated. The results showed that the absorbance of the solution increased with buffer volume up to 3 ml, and remained constant above that. Thus, 5 ml of citrate buffer pH 4.5 was added to the sample solutions to maintain the pH at 4.5.

Effect of $TBAHSO_4$

Ternary salts, such as tetrabutyl ammonium hydrogen sulfate $TBAHSO_4$, associate with anionic dyes to form ion-associates and are used in solvent extraction system [13]. Therefore, the effect of $TBAHSO_4$ concentration on the performance of the extraction system in the concentration range of 1×10^{-4}-6×10^{-4} M was studied. It was observed that the absorbance was increased up to $TBAHSO_4$ concentration of 3×10^{-4} M and remained constant above that. The concentration of 4×10^{-4} M of $TBAHSO_4$ was selected as optimum.

Effect of Triton X-100 Concentration

The effect of Triton X-100 concentration on the performance of the extraction system was studied. The variation of absorbance as a function of Triton X-100 concentration is shown in Fig. 3. Quantitative extraction was observed when the Triton X-100 concentration was above 0.020 M. The optimum surfactant concentration of 0.025 M of Triton X-100 was selected in order to achieve the optimal analytical signal in conjunction with the highest possible extraction efficiency.

Effect of Some Additives

The effect of electrolytes and some additives on the cloud point behavior of non-ionic surfactants was studied. It was observed that the presence of phenol, benzoic acid and xylenes decrease the cloud point temperature, resulting in a more efficient extraction [14,15]. Therefore, the effect of KCl and Na_2SO_4 as electrolyte, and benzoic acid as an additive, was studied. The results showed that when concentration of KCl is increased, the absorbance of solution was almost constant and benzoic acid showed a very negative effect on the cloud point extraction of amaranth, and the obtained solution by dissolving the surfactant rich phase in ethanol was colorless. However, Na_2SO_4 showed a slight increase on the absorbance of solution and the efficiency of the extraction. Therefore, the effect of its concentration in the range of 0.00-0.10 M was studied. It was found that the absorbance was increased up to Na_2SO_4 concentration of 0.07 M and remained constant above that. The concentration of 0.08 M of Na_2SO_4 was selected as optimum.

Effects of Equilibration Temperature and Time

Two important parameters in cloud point extraction are incubation time and equilibration temperature. Therefore, the

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effect of equilibration temperature in the range of 65-90 °C was studied. It was found that the extraction efficiency reaches maximum above 75 °C. So, an equilibration temperature of 80 °C was chosen for the analysis. The dependence of extraction efficiency upon equilibration time was also studied for a time interval of 15-40 min. Maximum extraction efficiency was observed above 30 min. Accordingly, an incubation time of 30 min was chosen for use in the next experiments.

Interference Studies

Various amounts of foreign ions and other dyes such as alizarin S, Pancio 4R, Eosin, Methyl red and Neutral red were added to the solutions containing 400 ng ml^{-1} of amaranth and the general procedure was followed. The tolerance limit was defined as the amount of foreign species causing a change in the absorbance of less than ±5%. The results presented in Table 1 show that very good selectivity is achieved.

Analytical Performance

Calibration graph was obtained using the general procedure for different concentrations of amaranth under the optimized experimental conditions. The calibration graph was linear in the range of 20-1600 ng ml^{-1} of amaranth in the initial aqueous solution. The equation of the regression line was $A = 7 \times 10^{-4} C + 0.0232$, where C is concentration amaranth in ng ml^{-1}. The correlation coefficient (r) was 0.9993 (n = 12). The relative standard deviation for 100 and 1000 ng ml^{-1} of amaranth was 4.2 and 1.4% (n = 10), respectively. The limit of detection of the method based on three times the standard deviation of the blank ($3S_b$) was 13 ng ml^{-1} (n = 10) of amaranth.

Applications

The method developed was applied to the determination of amaranth concentrations in beverage and jelly samples purchased from local market. For this purpose, jelly samples were dissolved in warm water and diluted to appropriate volume with water in a volumetric flask (jelly 1: 2.036 g in 25 ml water, jelly 2:2.004 g in 10 ml water and jelly 3:0.5 g in 10 ml water). 2 ml of jelly and beverage sample solutions were treated under the general procedure for the preconcentration and determination of amaranth. The recovery tests were performed by spiking amaranth to the samples. The results summarized in Table 2 show the good reliability of the method. The low detection limit allows the accurate determination of colorants in food samples.

CONCLUSIONS

A simple and sensitive cloud point extraction procedure was developed for preconcentration and determination of amaranth. The proposed method needs no pretreatment procedure for the extraction of colorants from food and offers a combination of sensitivity, selectivity, simplicity and relatively short time of analysis. The detection limit achieved is better than some of the previously reported methods [2,11-14]. The applicability was verified by the determination of amaranth present in several commercial food products and soft

Table 1. The Effect of Foreign Species on the Determination of 400 ng ml^{-1} Amaranth

Foreign species	Tolerance ratio (Foreign species conc./amaranth conc.)
Co^{2+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Ca^{2+}, Zn^{2+}, Cr^{3+}, NH_4^+, PO_4^{3-}, K^+, ClO_4^-, I^-, NO_3^-, Mn^{2+}	1000
Ba^{2+}, Br^-, Mg^{2+}	500
NO_2^-, Cd^{2+}, Pb^{2+}	250
Alizarin red S	50
Pancio 4R, Eosin	10
Methyl red	5
Neutral red	2

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Table 2. Determination of Amaranth in Beverage and Jelly Samples

Sample	Amaranth added (ng ml^{-1})	Amaranth found (ng ml^{-1})[a]	Recovery (%)
Beverage[b]	-	123.2 ± 2.0	-
	200	320.0 ± 3.0	98.4
	400	525.5 ± 3.0	100.6
Jelly 1[c]	-	209.0 ± 2.0	-
	200	408.1 ± 3.0	99.5
	400	602.8 ± 4.0	98.5
Jelly 2[d]	-	127.7 ± 1.0	-
	200	325.9 ± 2.0	99.1
	400	525.5 ± 4.0	99.5
Jelly 3[e]	-	320.0 ± 2.0	-
	200	506.4 ± 3.0	93.2
	400	717.8 ± 5.0	99.5

[a] $x \pm ts/\sqrt{n}$ at 95% confidence (n = 5). Amount of amaranth in beverage was 3.1 μg ml^{-1}. [b] Amount of amaranth in Jelly 1 was 64.1 μg g^{-1}. [c] Amount of amaranth in Jelly 2 was 31.9 μg g^{-1}. [d] Amount of amaranth in Jelly 3 was 160.0 μg g^{-1}.

drink samples.

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